CHEMISTRY
A EUROPEAN JOURNAL

Supporting Information

Solvent Effects on Environmentally Coupled Hydrogen Tunnelling During Catalysis by Dihydrofolate Reductase from *Thermotoga maritima*

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Supporting Information

TABLES

	Viscosity / mPa.s		Dielectric constant	
Solvent composition	20 ºC	40 ºC	20 ºC	40 ºC
No cosolvent	1	0.66	79	73.1
17 % methanol	1.25	0.8	75	69.3
33 % methanol	1.5	1	65	60
50 % methanol	1.6	1.1	58	53.6
17 % ethanol	1.45	1	71	65
33 % ethanol	2	1.5	60.7	56
50 % ethanol	2.3	1.8	52	48
17 % isopropanol	1.5	1	69.8	64
33 % isopropanol	2.1	1.6	59.4	55
50 % isopropanol	2.6	1.9	50	47
17 % ethylene glycol	1.6	1	76	66
33 % ethylene glycol	2.4	1.6	70	60
50 % ethylene glycol	3.4	2.4	65	54
17 % glycerol	1.8	1.1	74.4	67.5
33 % glycerol	3.4	2	67.7	62.7
50 % glycerol	8	4	62	57.3
17 % sucrose	1.8	1.1	76.5	68.5
30 % sucrose	3.4	1.9	72.6	65.6
17 % tetrahydrofuran	1	0.7	66.7	60
33 % tetrahydrofuran	1.2	0.85	54.1	47
50 % tetrahydrofuran	1.4	1	40	37

Table S1. Viscosity and dielectric constant of the solvent mixtures used in this study. Values were obtained from various literature sources.[1-9]

	20 ºC				40 ºC	
Solvent composition	k_{cat} / s^{-1}	KIE (k_{cat})	k_H / s^{-1}	KIE (k_H)	k_H / s^{-1}	KIE (k_H)
No cosolvent	0.075 ± 0.002	2.80 ± 0.01	0.122 ± 0.003	4.52 ± 0.20	0.488 ± 0.010	4.21 ± 0.27
17 % methanol	0.074 ± 0.003	2.22 ± 0.09	0.116 ± 0.002	4.48 ± 0.20	0.450 ± 0.006	3.45 ± 0.06
33 % methanol	0.042 ± 0.002	1.73 ± 0.10	0.086 ± 0.003	5.02 ± 0.04	0.402 ± 0.006	3.76 ± 0.04
50 % methanol	0.011 ± 0.002	1.31 ± 0.44	0.060 ± 0.008	5.26 ± 0.16	0.196 ± 0.009	3.76 ± 0.06
17 % ethanol	0.060 ± 0.002	2.14 ± 0.11	0.101 ± 0.001	4.61 ± 0.08	0.434 ± 0.003	4.27 ± 0.06
33 % ethanol	0.037 ± 0.005	2.09 ± 0.16	0.76 ± 0.001	4.39 ± 0.06	0.272 ± 0.004	3.43 ± 0.08
50 % ethanol	0.028 ± 0.004	1.96 ± 0.16	0.046 ± 0.001	4.48 ± 0.06	0.156 ± 0.038	3.65 ± 0.16
17 % isopropanol	0.053 ± 0.002	2.28 ± 0.11	0.077 ± 0.001	2.68 ± 0.05	0.402 ± 0.002	4.25 ± 0.03
33 % isopropanol	0.028 ± 0.002	1.96 ± 0.12	0.071 ± 0.007	4.08 ± 0.10	0.259 ± 0.003	4.78 ± 0.04
50 % isopropanol	0.015 ± 0.001	1.91 ± 0.15	0.051 ± 0.007	4.03 ± 0.17	0.130 ± 0.006	4.89 ± 0.06
17 % ethylene glycol	0.065 ± 0.002	2.57 ± 0.09	0.119 ± 0.001	4.79 ± 0.05	0.490 ± 0.007	4.62 ± 0.09
33 % ethylene glycol	0.038 ± 0.002	2.29 ± 0.10	0.106 ± 0.002	5.35 ± 0.08	0.437 ± 0.002	5.09 ± 0.02
50 % ethylene glycol	0.014 ± 0.002	1.83 ± 0.35	0.078 ± 0.011	4.30 ± 0.14	0.292 ± 0.022	4.76 ± 0.14
17 % glycerol	0.061 ± 0.001	2.32 ± 0.19	0.154 ± 0.005	3.63 ± 0.13	0.580 ± 0.027	3.05 ± 0.10
33 % glycerol	0.053 ± 0.006	2.04 ± 0.28	0.207 ± 0.015	2.96 ± 0.12	0.779 ± 0.028	2.62 ± 0.18
50 % glycerol	0.019 ± 0.001	1.19 ± 0.10	0.301 ± 0.036	2.42 ± 0.14	1.112 ± 0.057	2.28 ± 0.16
17 % sucrose	0.074 ± 0.003	2.64 ± 0.05	0.155 ± 0.007	3.48 ± 0.16	0.624 ± 0.015	3.52 ± 0.04
30 % sucrose	0.074 ± 0.001	2.32 ± 0.07	0.139 ± 0.003	3.34 ± 0.15	0.501 ± 0.003	3.15 ± 0.04
17 % tetrahydrofuran	0.025 ± 0.002	3.36 ± 0.18	0.031 ± 0.001	4.09 ± 0.02	0.113 ± 0.028	4.32 ± 0.25
33 % tetrahydrofuran	0.0092 ± 0.0010	2.89 ± 0.16	0.012 ± 0.001	3.14 ± 0.23	0.014 ± 0.001	2.12 ± 0.07
50 % tetrahydrofuran	0.0024 ± 0.0003	2.97 ± 0.28	0.0029 ± 0.0010	2.92 ± 0.47	0.0086 ± 0.0014	3.34 ± 0.21

Table S2. Kinetic parameters for the reaction of NADPH and H_2F catalysed by TmDHFR in the presence of cosolvents.

	k_H / s^{-1}				k_D / s^{-1}			
T / ºC	0 %[1]	17 %	33 %	50 %	0 %[1]	17 %	33 %	50 %
7	0.044 ± 0.001[2]	0.044 ± 0.001	0.020 ± 0.003	0.018 ± 0.001	0.0066 ± 0.0002[2]	0.0077 ± 0.0005	0.0051 ± 0.0008	0.005 ± 0.001
10	0.06 ± 0.003[2]	0.053 ± 0.002	0.042 ± 0.004	0.021 ± 0.001	0.0097 ± 0.001[2]	0.0092 ± 0.0009	0.0084 ± 0.0013	0.005 ± 0.001
15	0.087 ± 0.002[2]	0.078 ± 0.002	0.065 ± 0.003	0.047 ± 0.004	0.016 ± 0.001[2]	0.015 ± 0.0003	0.012 ± 0.002	0.010 ± 0.001
20	0.122 ± 0.003	0.116 ± 0.002	0.086 ± 0.003	0.060 ± 0.008	0.027 ± 0.001	0.024 ± 0.005	0.017 ± 0.001	0.011 ± 0.001
25	0.169 ± 0.002	0.165 ± 0.003	0.140 ± 0.006	0.100 ± 0.003	0.042 ± 0.003	0.043 ± 0.002	0.037 ± 0.002	0.024 ± 0.005
30	0.242 ± 0.005	0.238 ± 0.008	0.206 ± 0.004	0.119 ± 0.006	0.06 ± 0.002	0.066 ± 0.001	0.055 ± 0.001	0.029 ± 0.004
35	0.341 ± 0.006	0.329 ± 0.009	0.289 ± 0.005	0.158 ± 0.004	0.082 ± 0.005	0.093 ± 0.009	0.081 ± 0.002	0.041 ± 0.002
40	0.488 ± 0.010	0.450 ± 0.006	0.402 ± 0.006	0.196 ± 0.009	0.116 ± 0.007	0.130 ± 0.007	0.107 ± 0.004	0.052 ± 0.002
45	0.668 ± 0.015	0.652 ± 0.057	0.579 ± 0.029	0.233 ± 0.019	0.161 ± 0.003	0.166 ± 0.007	0.143 ± 0.012	0.064 ± 0.010
50	0.932 ± 0.036	0.844 ± 0.073	0.780 ± 0.018	0.372 ± 0.026	0.245 ± 0.011	0.245 ± 0.005	0.208 ± 0.015	0.095 ± 0.011
55	1.253 ± 0.057				0.336 ± 0.015			
60	1.654 ± 0.093				0.444 ± 0.019			
65	2.12 ± 0.159				0.575 ± 0.013			

[1] Data from Maglia and Allemann (2003)[10] [2] Data measured at 6 ºC, 11 ºC and 16ºC.

Table S3. Temperature dependence of the pre-steady-state kinetic parameters for the reaction of NADPH and H_2F catalysed by TmDHFR in the presence of varying concentrations of methanol.

	k_H / s^{-1}				k_D / s^{-1}			
T / ºC	0 %[1]	17 %	33 %	50 %	0 %[1]	17 %	33 %	50 %
6	0.044 ± 0.001	0.058 ± 0.006	0.076 ± 0.007	0.103 ± 0.004	0.0066 ± 0.0002	0.012 ± 0.001	0.017 ± 0.003	0.028 ± 0.002
8	nd	0.061 ± 0.001	0.096 ± 0.001	0.125 ± 0.007	nd	0.012 ± 0.001	0.023 ± 0.002	0.036 ± 0.001
10	0.06 ± 0.003[2]	0.072 ± 0.005	0.107 ± 0.007	0.138 ± 0.009	0.0097 ± 0.001[2]	0.015 ± 0.001	0.030 ± 0.003	0.048 ± 0.010
12.5	nd	0.089 ± 0.001	0.132 ± 0.006	0.173 ± 0.005	nd	0.021 ± 0.001	0.038 ± 0.002	0.069 ± 0.002
15	0.087 ± 0.002[2]	0.103 ± 0.010	0.147 ± 0.009	0.200 ± 0.018	0.016 ± 0.001[2]	0.028 ± 0.004	0.050 ± 0.004	0.081 ± 0.005
17.5	nd	0.126 ± 0.003	nd	nd	nd	0.035 ± 0.004	nd	nd
20	0.122 ± 0.003	0.154 ± 0.005	0.207 ± 0.015	0.301 ± 0.036	0.027 ± 0.001	0.047 ± 0.003	0.070 ± 0.007	0.124 ± 0.011
25	0.169 ± 0.002	0.208 ± 0.021	0.302 ± 0.009	0.414 ± 0.068	0.042 ± 0.003	0.066 ± 0.006	0.112 ± 0.007	0.182 ± 0.02
30	0.242 ± 0.005	0.291 ± 0.033	0.424 ± 0.013	0.607 ± 0.020	0.06 ± 0.002	0.094 ± 0.011	0.152 ± 0.014	0.287 ± 0.027
35	0.341 ± 0.006	0.408 ± 0.035	0.556 ± 0.026	0.791 ± 0.054	0.082 ± 0.005	0.130 ± 0.013	0.209 ± 0.037	0.362 ± 0.029
40	0.488 ± 0.010	0.580 ± 0.027	0.779 ± 0.028	1.112 ± 0.057	0.116 ± 0.007	0.190 ± 0.017	0.298 ± 0.052	0.487 ± 0.073
45	0.668 ± 0.015	0.788 ± 0.025	1.100 ± 0.089	1.510 ± 0.091	0.161 ± 0.003	0.252 ± 0.028	0.394 ± 0.059	0.744 ± 0.060
50	0.932 ± 0.036	1.061 ± 0.019	1.571 ± 0.189	2.180 ± 0.128	0.245 ± 0.011	0.362 ± 0.045	0.597 ± 0.065	0.970 ± 0.127

[1] Data from Maglia and Allemann (2003)[10] [2] Data measured at 11 ºC and 16ºC.

Table S4. Temperature dependence of the pre-steady-state kinetic parameters for the reaction of NADPH and H_2F catalysed by TmDHFR in the presence of varying concentrations of glycerol.

	k_H / s^{-1}			k_D / s^{-1}		
T / ºC	0 %[1]	17 %	30 %	0 %[1]	17 %	30 %
6	0.044 ± 0.001	0.071 ± 0.005	0.066 ± 0.009	0.0066 ± 0.0002	0.019 ± 0.001	0.019 ± 0.001
10	0.06 ± 0.003[2]	0.082 ± 0.001	0.084 ± 0.006	0.0097 ± 0.001[2]	0.021 ± 0.002	0.025 ± 0.001
15	0.087 ± 0.002[2]	0.109 ± 0.001	0.106 ± 0.003	0.016 ± 0.001[2]	0.030 ± 0.004	0.032 ± 0.002
20	0.122 ± 0.003	0.155 ± 0.002	0.139 ± 0.002	0.027 ± 0.001	0.045 ± 0.007	0.042 ± 0.006
25	0.169 ± 0.002	0.230 ± 0.001	0.206 ± 0.006	0.042 ± 0.003	0.070 ± 0.013	0.060 ± 0.005
30	0.242 ± 0.005	0.326 ± 0.003	0.285 ± 0.010	0.06 ± 0.002	0.094 ± 0.016	0.092 ± 0.009
35	0.341 ± 0.006	0.459 ± 0.014	0.391 ± 0.008	0.082 ± 0.005	0.130 ± 0.002	0.126 ± 0.006
40	0.488 ± 0.010	0.624 ± 0.015	0.501 ± 0.003	0.116 ± 0.007	0.178 ± 0.007	0.159 ± 0.006
45	0.668 ± 0.015	0.838 ± 0.008	0.764 ± 0.026	0.161 ± 0.003	0.223 ± 0.012	0.222 ± 0.004
50	0.932 ± 0.036	1.096 ± 0.005	0.948 ± 0.044	0.245 ± 0.011	0.348 ± 0.042	0.293 ± 0.007
55	1.253 ± 0.057			0.336 ± 0.015		
60	1.654 ± 0.093			0.444 ± 0.019		
65	2.12 ± 0.159			0.575 ± 0.013		

[1] Data from Maglia and Allemann (2003)[10] [2] Data measured at 11 ºC and 16ºC.

Table S5. Temperature dependence of the pre-steady-state kinetic parameters for the reaction of NADPH and H_2F catalysed by TmDHFR in the presence of varying concentrations of sucrose.

T / ºC	KIE								
	0 %[1]	Methanol			Glycerol			Sucrose	
		17 %	33 %	50 %	17 %	33 %	50 %	17 %	30 %
6	6.67 ± 0.25[2]	5.74 ± 0.07[2]	3.95 ± 0.20[2]	3.73 ± 0.21[2]	4.93 ± 0.14	4.43 ± 0.20	3.64 ± 0.10	3.79 ± 0.11	3.55 ± 0.14
8	nd	nd	nd	nd	4.89 ± 0.12	4.15 ± 0.07	3.49 ± 0.07	nd	nd
10	6.19 ± 0.71[2]	5.75 ± 0.10	4.98 ± 0.19	3.93 ± 0.03	4.71 ± 0.10	3.59 ± 0.12	2.87 ± 0.23	3.93 ± 0.10	3.42 ± 0.09
12.5	nd	nd	nd	nd	4.21 ± 0.06	3.42 ± 0.07	2.51 ± 0.04	nd	nd
15	5.44 ± 0.36[2]	5.27 ± 0.03	5.37 ± 0.18	4.90 ± 0.15	3.63 ± 0.17	2.96 ± 0.11	2.46 ± 0.11	3.60 ± 0.12	3.28 ± 0.06
17.5	nd	nd	nd	nd	3.63 ± 0.13	nd	nd	nd	nd
20	4.52 ± 0.2	4.88 ± 0.20	5.02 ± 0.04	5.26 ± 0.16	3.29 ± 0.08	2.96 ± 0.12	2.42 ± 0.15	3.48 ± 0.16	3.34 ± 0.15
25	4.02 ± 0.29	3.87 ± 0.05	3.80 ± 0.06	4.20 ± 0.20	3.17 ± 0.14	2.71 ± 0.07	2.27 ± 0.20	3.29 ± 0.18	3.45 ± 0.09
30	4.03 ± 0.16	3.58 ± 0.03	3.76 ± 0.03	4.12 ± 0.15	3.10 ± 0.16	2.79 ± 0.10	2.12 ± 0.10	3.48 ± 0.17	3.11 ± 0.10
35	4.16 ± 0.26	3.53 ± 0.11	3.55 ± 0.04	3.86 ± 0.05	3.13 ± 0.13	2.66 ± 0.18	2.18 ± 0.11	3.52 ± 0.04	3.10 ± 0.05
40	4.21 ± 0.27	3.47 ± 0.06	3.76 ± 0.04	3.76 ± 0.06	3.05 ± 0.10	2.62 ± 0.18	2.28 ± 0.16	3.52 ± 0.04	3.16 ± 0.04
45	4.15 ± 0.12	3.92 ± 0.10	4.05 ± 0.10	3.62 ± 0.17	3.13 ± 0.12	2.79 ± 0.17	2.03 ± 0.10	3.76 ± 0.06	3.43 ± 0.04
50	3.80 ± 0.23	3.44 ± 0.09	3.75 ± 0.07	3.90 ± 0.14	2.93 ± 0.12	2.63 ± 0.16	2.25 ± 0.14	3.1 ± 0.12	3.24 ± 0.05
55	3.73 ± 0.24								
60	3.73 ± 0.26								
65	3.69 ± 0.29								

[1] Data from Maglia and Allemann (2003)[10] [2] Data measured at 6 ºC, 11 ºC and 16 ºC.

Table S6. Temperature dependence of the kinetic isotope effects for the reaction of NADPH and H_2F catalysed by TmDHFR in the presence of varying concentrations of methanol.

Cosolvent	E_a^H / kJ.mol^{-1}	E_a^D / kJ.mol^{-1}	ΔE_a / kJ.mol^{-1}	A_H / s^{-1}	A_D / s^{-1}	A_H/A_D	KIE
None	53.5 ± 0.4	56.0 ± 0.8	2.5 ± 1.0	(4.11 ± 0.68) x 10^{8}	(2.67 ± 0.86) x 10^{8}	1.56 ± 0.47	3.9 ± 0.2
17 % glycerol	52.5 ± 0.4	54.3 ± 0.9	1.8 ± 1.0	(3.31 ± 0.55) x 10^{8}	(2.14 ± 0.71) x 10^{8}	1.55 ± 0.58	3.1 ± 0.1
33 % glycerol	52.1 ± 1.1	54.2 ± 1.3	2.1 ± 1.7	(3.97 ± 1.68) x 10^{8}	(3.26 ± 1.70) x 10^{8}	1.22 ± 0.82	2.7 ± 0.1
50 % glycerol	51.7 ± 0.8	54.4 ± 1.3	2.7 ± 1.5	(4.69 ± 1.39) x 10^{8}	(6.10 ± 3.03) x 10^{8}	0.77 ± 0.45	2.2 ± 0.1
17 % sucrose	48.9 ± 1.1	50.8 ± 1.2	1.9 ± 1.7	(8.89 ± 4.00) x 10^{7}	(5.31 ± 2.60) x 10^{7}	1.68 ± 1.12	3.6 ± 0.2
30 % sucrose	46.5 ± 1.2	47.7 ± 1.1	1.2 ± 1.6	(2.94 ± 1.46) x 10^{7}	(1.46 ± 0.62) x 10^{7}	2.02 ± 1.32	3.3 ± 0.2

Table S7. Activation energies and Arrhenius prefactors for the region of TmDHFR catalysis showing temperature-independent KIEs, in the presence of varying concentrations of glycerol and sucrose.

Cosolvent	E_a^H / kJ.mol^{-1}	E_a^D / kJ.mol^{-1}	ΔE_a / kJ.mol^{-1}	A_H / s^{-1}	A_D / s^{-1}	A_H/A_D
None	49.9 ± 1.7	69.2 ± 3.7	18.5 ± 6.7	(7.66 ± 0.50) x 10^{7}	(3.18 ± 0.34) x 10^{10}	(2.41 ± 0.30) x 10^{-3}
17 % glycerol	48.7 ± 2.1	70.1 ± 3.4	21.4 ± 4.0	(7.14 ± 0.35) x 10^{7}	(1.42 ± 0.80) x 10^{11}	(5.03 ± 0.37) x 10^{-4}
33 % glycerol	48.4 ± 4.4	78.0 ± 3.8	29.6 ± 5.8	(9.05 ± 0.92) x 10^{7}	(7.09 ± 0.39) x 10^{12}	(1.28 ± 0.15) x 10^{-5}
50 % glycerol	51.2 ± 3.9	91.7 ± 3.1	40.4 ± 5.0	(4.04 ± 0.34) x 10^{8}	(3.98 ± 0.15) x 10^{15}	(1.01 ± 0.09) x 10^{-7}

Table S8. Activation energies and Arrhenius prefactors for the region of TmDHFR catalysis showing temperature-dependent KIEs, in the presence of varying concentrations of glycerol.

FIGURES

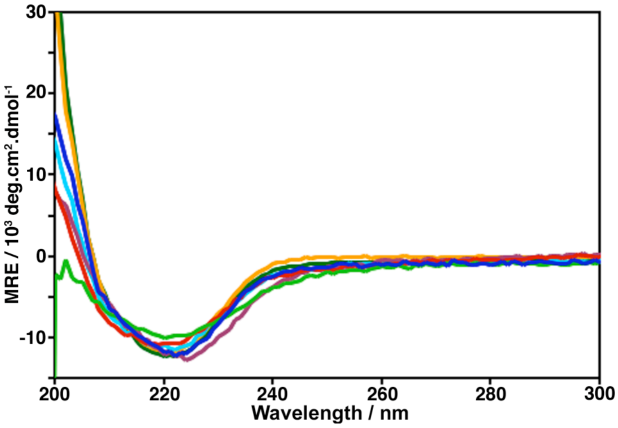

Figure S1. CD spectra of TmDHFR (10 µM) at 20 °C in the presence of 50% of organic cosolvents. Dark green = no cosolvent, light green = tetrahydrofuran, yellow = sucrose, orange = glycerol, red = ethylene glycol, maroon = isopropanol, dark blue = ethanol, and light blue = methanol.

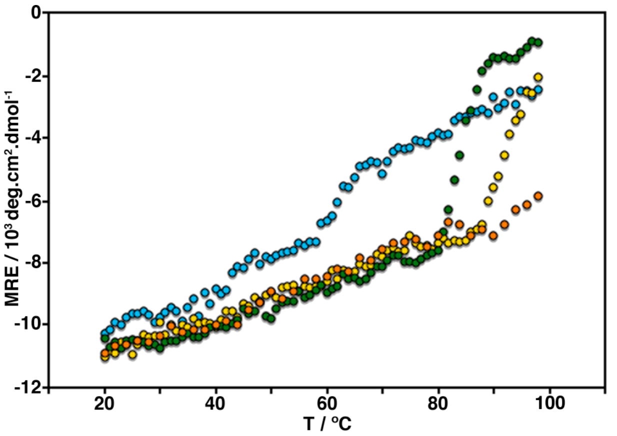

Figure S2. Temperature dependence of the CD signal at 222 nm of TmDHFR in the presence of 50% methanol (light blue), glycerol (orange), sucrose (yellow), or no cosolvent (dark green). Conditions as described for Figure S1.

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